

IL-10 Gene Polymorphisms and Their Association with Immune Traits in Four Rabbit Populations

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ABSTRACT: Interleukin-10 (IL-10) has been recently identified as a multifunctional cytokine, because of its close link with immunoregulation and anti-inflammatory responses. This study investigated the association of IL-10 genetic polymorphisms with the immune traits of New Zealand white rabbits (N-W), Fujian yellow rabbits (F-Y) and their reciprocal crosses (N-Y and Y-N, respectively). SNPs on five exons of the IL-10 gene were genotyped in 204 healthy rabbits via PCR-SSCP and DNA sequencing. Two SNPs (A1435G and G1519A, both were synonymous mutations) and six genotypes (AA, BB, CC, AB, AC and BC) were found on exon 3 and one SNP (T base insertion between loci 2532 and 2533, which caused a frameshift mutation), and three genotypes (OO, TT and TO) were present on exon 4. Allele A was the most frequent allele on exon 3 (from 0.548 to 0.771), whereas O was the most frequent on exon 4 (from 0.808 to 0.968). These four populations were all in Hardy-Weinberg equilibrium on both exon3 and exon4. Association analysis between polymorphisms and immune parameters showed that SNPs on exon 3 were significantly associated with immune traits, while SNP on exon 4 may not significantly affect immune traits, but the mechanism is yet to be further studied.

KEY WORDS: exon, IL-10 gene, immune parameters, PCR-SSCP, polymorphism.

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Interleukin 10 (IL-10) is a special cytokine that was first discovered in mice in 1989 by Fiorentino of the DNAX Research Institute. IL-10 inhibits the activation of monocyte/macrophage systems and the synthesis of monokine and inflammatory factors. Except for Th2 cells, T cell subsets, monocytes/macrophages, mast cells, keratinocytes, eosinophils, epithelial cells and natural killer cells synthesize IL-10 [18]. IL-10 has important immunoregulatory activity [7, 21–23] and is closely related to the occurrence of parasitic diseases [1, 17]. Further studies found that single nucleotide polymorphisms (SNPs) of the IL-10 gene directly influence the expression of IL-10 *in vivo*; thus, they are closely related to the susceptibility, severity and the developmental process of diseases [3, 11, 16, 24, 27]. Therefore, studies on the genetic variation characteristics of rabbit IL-10 gene have important practical significance.

In addition to IL-10, Immunoglobulin G (IgG), Interferon γ (IFN- γ) and White blood cells (WBCs) can also reflect the immune performance. IgG, an immunoglobulin with the highest levels in the serum, has an important function in the immune system. Its continual role lasts for a long time, so IgG has a major function in antibody-mediated defense. IFN- γ is another important cytokine that has a variety of

biological activities, such as broad-spectrum anti-viral, anti-cell proliferation and immunoregulatory activity. WBCs are closely related to immune organs and immune tissues, and thus, they participate in cellular immunity.

Studies related to rabbit IL-10 gene exons are rare. In this study, polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) method was used to detect the SNPs on 5 exons of the IL-10 gene in 4 rabbit populations to compare and analyze their genetic differences. Furthermore, we determined the relevant immune parameters of these 4 populations and conducted association analyses between polymorphisms and immune parameters to provide a theoretical foundation for further studies on the association between rabbit IL-10 gene and disease resistance.

MATERIALS AND METHODS

Sample collection: All the subjects involved in the study, including N-W (n=52), F-Y (n=66), N-Y (n=24) and Y-N (n=62), were randomly drawn from the Jinling Rabbit Farm in Jiangsu Province. The 204 healthy rabbits were all born within the same period and reared under the same conditions. Blood samples were collected when they were 3 months old. After 12 hr of fasting, a 9 ml blood sample was collected from the ear vein of each rabbit. Up to 5 ml was placed into procoagulant collection tubes and 4 ml into the anticoagulant blood collection tubes. The routine blood index for WBC was measured using a routine protocol. Genomic DNA was extracted from anticoagulant whole blood using the phenol/chloroform method and then stored at -20°C . Procoagulant blood was centrifuged for 10 min at 3,000 rpm, and then, the serum was stored at -20°C for the determination of immune

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Table 1. Primer sequences

Primers	Primer sequences (5'-3')	Fragment size (bp)	Annealing temperature (°C)
1	F:cacaagcgggactcgtaga R:ggacaggaggcatcact	225	58
2	F:aacgaatggctccagcacta R:cgcctccctcccttaac	288	58
3	F:tatccaagccttgtcgg R:agaagcgtctgctactca	162	63
4	F:tgacagccaaggtcattaaca R:cggggagcagtcatttagaa	255	63
5	F:cctcggagtgaagatgcttag R:gatcgggagctgaggtatca	290	58

F represents for forward primer, R represents for reverse primer.

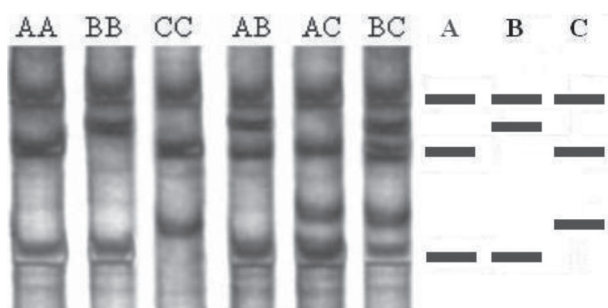


Fig. 1. SSCP results of different genotypes on exon 3. The left figure is the electropherogram of 6 genotypes on exon 3, and the right one is the band of A, B and C.

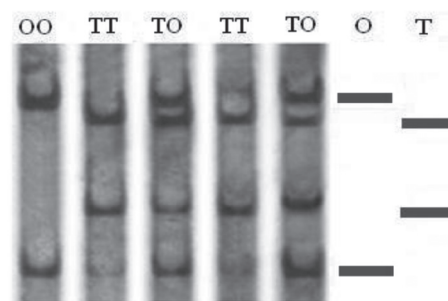


Fig. 2. SSCP results of different genotypes on exon 4. The left figure is the electropherogram of three genotypes on exon 4, and the right one is the band of O and T.

parameters, such as IgG, IL-10 and IFN- γ . Repeated freezing and thawing was avoided.

PCR-SSCP detection and genotype determination: Five pairs of specific primers were designed according to the rabbit IL-10 gene sequence published in *GenBank* (Accession number: DQ437508.2) to amplify the 5 exons. The amplified products were detected using the SSCP method and sent to Shanghai Sangon Biological Engineering Co., Ltd. for bidirectional sequencing. The sequencing results were compared through Align and DNASTar software to search for mutation sites.

The primer sequences are shown in Table 1.

PCR reactions were carried out in 20 μ l reaction mixtures containing 2 μ l of 10 \times PCR buffer (Mg²⁺ plus), 0.4 μ l of dNTPs (10 mmol/l), 1 μ l of each primer (10 pmol/l), 0.2 μ l of Taq DNA polymerase (5 U/ μ l), 1 μ l of DNA template (100 ng/ μ l) and 14.4 μ l of ddH₂O. The PCR amplification conditions were as follows: 40 cycles of 94°C for 40 sec, annealing at the appropriate temperature for 40 sec, extension at 72°C for 40 sec, an initial denaturation at 95°C for 5 min and final extension at 72°C for 10 min.

Determination of immune parameters: The determination of immune parameters was based on the method by Zhangjian *et al.* [26]. The WBC counts were detected using CD-1200-type fully automatic blood analyzer (Abbott, Chicago, IL, U.S.A.). Determination of the immune parameters, such as IgG, IL-10 and IFN- γ , was in accordance with the instruc-

tions of the enzyme-linked immunosorbent assay kit (Bogoo Bio, Shanghai, China).

Association analysis between polymorphisms and immune parameters: Association analysis between polymorphisms and immune parameters of different genotypes was performed to ascertain which genotypes are closely associated with immune traits. To ensure the accuracy and reliability of the data analysis, we excluded the rabbits with genotype counts of 0 or 1.

Statistical analysis: Genotypic and allelic frequencies were counted directly. The Hardy-Weinberg equilibrium was tested via χ^2 goodness of fit statistic using the following equation: $\chi^2 = \sum(O - E)^2/E$, where O stands for observed data and E stands for expected data. Population genetic diversity parameters, including polymorphism information content (PIC) and expected heterozygosity (*He*), were calculated using the method previously described by Nei *et al.* [15]. Sequence alignment was determined after using the DNASTar program (DNASTAR Co., Ltd., Madison, WI, U.S.A.). Amino acid sequence alignment was analyzed by ClustalW2 online software (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Gene functional domains were predicted by online software CDD (<http://www.ncbi.nlm.nih.gov/Structure/lexington/lexington.cgi>). Pairwise linkage disequilibrium (LD) between SNPs was calculated by r^2 value using SHEsis online software (<http://analysis.bio-x.cn/myAnalysis.php>) [20]. SPSS 13.0 statistical software (IBM Co., Ltd., Armonk,

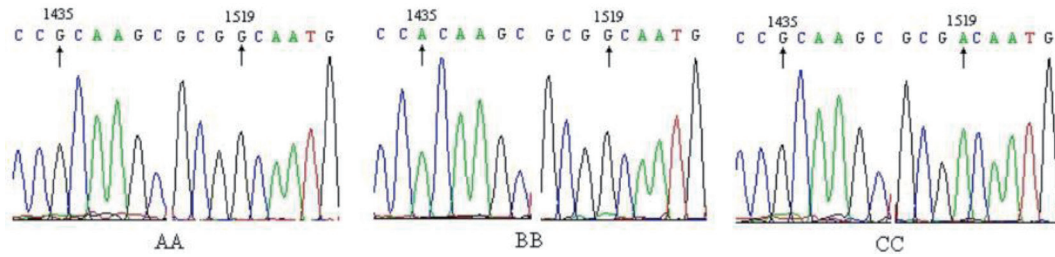


Fig. 3. Sequencing results of AA, BB and CC genotypes on exon 3. What the arrows point to are bases at loci 1435 and 1519, respectively.

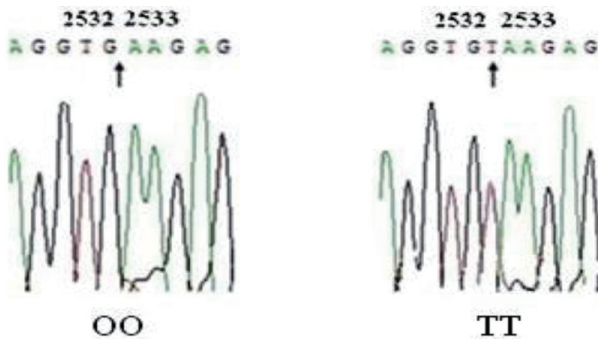


Fig. 4. Sequencing results of OO and TT genotypes on exon 4. The arrow points to the T base insertion between loci 2532 and 2533. Genotype OO has no T insertion, while genotype TT dose, in this locus.

NY, U.S.A.) was used to test the significance and for least significant difference multiple comparisons. The results are presented as “mean value \pm standard error.” Differences with $P < 0.05$ were considered statistically significant.

RESULTS

Analysis of PCR-SSCP: Target fragments were amplified with the designed 5 pairs of primers. PCR amplification results showed that the bands had good specificity and the fragment size was consistent with the theoretical value. After the detection of SSCP, no SNPs were found on exon 1, 2 or 5. Exon 3 contained three alleles and 6 genotypes (AA, BB, CC, AB, AC and BC; Fig. 1). Exon 4 had 2 alleles and three genotypes (OO, TT and TO; Fig. 2). The sequencing results showed that two mutations occurred on exon 3, A1435G and G1519A (Fig. 3), which were synonymous mutations that did not cause any amino acid changes. Exon 4 contained a T base insertion between loci 2532 and 2533 (Fig. 4) that caused a frameshift mutation. Amino acid sequences before and after T base insertion mutation show that the quantity of amino acids coded by the mutated nucleotide sequence is reduced from 178 to 142, which means that the T base insertion early terminates the amino acid coding. The prediction result of IL-10 gene functional domains shows that the domains are within amino acids 4 to 131 (figures not shown).

Linkage disequilibrium: $r^2 > 0.33$ means that there is strong

linkage disequilibrium between 2 SNPs [2]. The results of pairwise linkage disequilibrium between SNPs showed that SNPs A1435G and G1519A were not in strong linkage disequilibrium ($r^2 = 0.026 < 0.33$), neither did G1519A and 2532T2533 ($r^2 = 0.027 < 0.33$). While, SNPs A1435G and 2532T2533 were strongly linked ($r^2 = 0.766 > 0.33$), which indicated that this region might be inherited as a unit.

Genetic distribution of exon 3 in the 4 populations: The genetic distribution of exon 3 in the 4 rabbit populations is shown in Table 2. The CC genotype was the rarest among the 4 populations with one individual in N-W. The frequency of allele A was much higher than that of alleles B and C, so A is the most frequent allele. The heterozygosity of N-W and F-Y was higher than that of the other 2 populations. According to the *PIC* classification ($PIC < 0.25$, low polymorphism; $0.25 < PIC < 0.5$, medium polymorphism; $PIC > 0.5$, high polymorphism), N-W and F-Y have high polymorphism, whereas the other 2 populations both have medium polymorphic levels. The Hardy–Weinberg equilibrium test indicated that these 4 populations were all in equilibrium.

Genetic distribution of exon 4 in the 4 populations: The genetic distribution of exon 4 in the 4 rabbit populations is shown in Table 3. In all populations, genotype TT had the least proportion with only one individual in the N-W and the F-Y populations. The frequency of allele O was much higher than that of allele T, so O is the most frequent allele. Considering the 4 populations only have 2 or 3 genotypes with the OO genotype as the most frequent, the *He* and the *PIC* (median or low polymorphism) of exon 4 are both relatively low. The Hardy–Weinberg equilibrium test indicated that these four populations were all in equilibrium.

Association of exon 3 polymorphism with immune parameters: The immune parameters of the different exon 3 genotypes in the 4 populations are shown in Table 4. The levels of IgG, IL-10 and IFN- γ were not significantly different among the genotypes in the N-W population ($P > 0.05$). Rabbits with genotypes AA, AB and AC all had extremely significantly higher WBC counts than those with genotypes BB and BC ($P < 0.01$) with no significant differences among rabbits with genotypes AA, AB and AC and between rabbits with genotypes BB and BC ($P > 0.05$).

In the Y-N population, no significant differences in IFN- γ levels and WBC counts were observed among the genotypes ($P > 0.05$). Rabbits with genotype AB had the highest IgG

Table 2. Genetic distribution of exon 3 in the 4 populations

Populations	Sample counts	Genotypic frequencies					Allelic frequencies			<i>He</i>	<i>PIC</i>	χ^2	
		AA	BB	CC	AB	AC	BC	A	B				C
N-W	52	0.269 (14)	0.115 (6)	0.019 (1)	0.269 (14)	0.288 (15)	0.038 (2)	0.548	0.269	0.163	0.601	0.538	3.19 (5)
Y-N	62	0.484 (30)	0.048 (3)	0 (0)	0.371 (23)	0.016 (1)	0.081 (5)	0.677	0.275	0.048	0.464	0.392	3.65 (4)
N-Y	24	0.583 (14)	0.042 (1)	0 (0)	0.375 (9)	0 (0)	0 (0)	0.771	0.229	0	0.353	0.291	1.05 (2)
F-Y	66	0.561 (37)	0.045 (3)	0 (0)	0.318 (21)	0.076 (5)	0 (0)	0.758	0.204	0.038	0.589	0.557	1.32 (3)

The numbers in brackets in the genotypic frequencies are genotype counts, and the numbers in brackets in the χ^2 values are the degrees of freedom. $\chi^2_{(2)0.05}=5.99$, $\chi^2_{(3)0.05}=7.81$, $\chi^2_{(4)0.05}=9.49$, $\chi^2_{(5)0.05}=11.07$. $0.25 < PIC < 0.5$ means medium polymorphism, $PIC > 0.5$ means high polymorphism.

Table 3. Genetic distribution of exon 4 in the 4 populations

Populations	Sample counts	Genotypic frequencies			Allelic frequencies		<i>He</i>	<i>PIC</i>	χ^2
		OO	TT	TO	O	T			
N-W	52	0.635 (33)	0.019 (1)	0.346 (18)	0.808	0.192	0.310	0.262	0.47 (2)
Y-N	62	0.935 (58)	0 (0)	0.065 (4)	0.968	0.032	0.062	0.060	0 (1)
N-Y	24	0.917 (22)	0 (0)	0.083 (2)	0.958	0.042	0.080	0.077	0 (1)
F-Y	66	0.909 (60)	0.015 (1)	0.076 (5)	0.947	0.053	0.073	0.070	0 (2)

The numbers in brackets in the genotypic frequencies are genotype counts, and the numbers in brackets in the χ^2 values are the degrees of freedom. $\chi^2_{(1)0.05}=3.84$, $\chi^2_{(2)0.05}=5.99$. $PIC < 0.25$ means low polymorphism, $0.25 < PIC < 0.5$ means medium polymorphism.

levels, but had no significant differences with rabbits with genotypes BB and BC ($P > 0.05$), but were significantly higher than those with genotype AA ($P < 0.05$). No significant differences were found among genotypes AA, BB and BC ($P > 0.05$). Rabbits with genotype BB had the highest IL-10 levels with no significant differences with those with genotype BC ($P > 0.05$), but were extremely significantly higher than those with genotypes AA and AB ($P < 0.01$). No significant differences were observed between the latter 2 genotypes ($P > 0.05$).

The immune parameters of the rabbits in the N-Y population did not significantly differ among the genotypes ($P > 0.05$).

The IFN- γ levels of the rabbits in the F-Y population did not significantly differ among the genotypes ($P > 0.05$). The rabbits with genotype AA had extremely significantly lower IgG levels than those with the other 3 genotypes ($P < 0.01$) with no significant differences among those with the latter 3 genotypes ($P > 0.05$). Rabbits with genotype BB had significantly higher IL-10 levels than those with the other three genotypes ($P < 0.05$) with no significant differences among those with the latter 3 genotypes ($P > 0.05$). Rabbits with genotype AC had the highest WBC counts with no significant differences with those with genotypes BB and AB ($P > 0.05$), but were significantly higher than those with genotype AA ($P < 0.05$). No significant differences were found among the latter 3 genotypes ($P > 0.05$).

Association of exon 4 polymorphism with immune parameters: The immune parameters of the different exon 4

genotypes in the 4 populations are shown in Table 5. The immune parameters of the genotypes in the N-W, Y-N and N-Y populations were not significantly different ($P > 0.05$). No significant differences in the levels of IgG, IL-10 and IFN- γ were found among the rabbits with different genotypes in the F-Y population ($P > 0.05$), but the WBC counts of the F-Y rabbits with genotype TO were significantly higher than those with genotype OO ($P < 0.05$).

DISCUSSION

An increasing number of researchers are focusing their attention on the study of IL-10, because of its important biological role. The human IL-10 gene has a large number of SNPs in the promoter sequence. Interestingly, these SNPs are closely related to susceptibility to some diseases [10]. In this study, we analyzed the polymorphism on all exons of the IL-10 gene using the PCR-SSCP method. We found 2 mutations and six genotypes on exon 3 and one mutation and three genotypes on exon 4. Rabbits with the genotype CC on exon 3 and the genotype TT on exon 4 were relatively few. We only found these 2 genotypes in the N-W and F-Y populations, possibly because the sample size of the other populations was too small or because these 2 genotypes are unique to the N-W and F-Y populations. Alleles A and O had the highest frequencies on exons 3 and 4, respectively.

He reflects the genetic consistency of a population, that is, a lower *He* indicates less genetic variations in the population and consequently lower genetic diversity. In this study, the

Table 4. Immune parameters of the different exon 3 genotypes in the 4 populations

Populations	Genotype (number)	IgG levels (g/l)	IL-10 levels (pg/ml)	IFN- γ levels (pg/ml)	WBC counts ($10^9/l$)
N-W	AA(14)	17.659 \pm 0.666	199.699 \pm 17.426	149.618 \pm 5.149	8.329 \pm 0.521 ^{ab}
	BB(6)	18.489 \pm 1.017	227.733 \pm 26.618	151.012 \pm 7.865	6.683 \pm 0.797 ^c
	AB(14)	17.120 \pm 0.666	234.579 \pm 17.426	147.605 \pm 5.149	7.979 \pm 0.521 ^{ab}
	CC(1)	16.531	154.090	130.587	6.136
	AC(15)	18.609 \pm 0.643	220.205 \pm 16.835	149.532 \pm 4.974	8.987 \pm 0.504 ^a
	BC(2)	18.691 \pm 1.762	154.303 \pm 46.104	169.370 \pm 13.622	6.400 \pm 1.380 ^c
Y-N	AA(30)	15.872 \pm 0.478 ^b	279.586 \pm 9.783 ^c	162.445 \pm 6.942	9.150 \pm 0.377
	BB(3)	15.124 \pm 1.513 ^{ab}	352.075 \pm 30.936 ^a	196.898 \pm 21.951	10.133 \pm 1.191
	AB(23)	17.464 \pm 0.546 ^a	257.694 \pm 11.173 ^c	170.610 \pm 7.928	9.765 \pm 0.430
	AC(1)	15.908	277.465	127.162	8.336
	BC(5)	16.403 \pm 1.172 ^{ab}	301.685 \pm 23.963 ^{ab}	165.992 \pm 17.003	8.720 \pm 0.923
N-Y	AA(14)	16.364 \pm 0.567	244.861 \pm 13.422	185.982 \pm 10.899	9.943 \pm 0.699
	BB(1)	16.383	268.173	185.174	11.756
	AB(9)	16.360 \pm 0.707	239.803 \pm 16.740	162.906 \pm 13.593	10.256 \pm 0.872
F-Y	AA(37)	18.707 \pm 0.682 ^c	260.114 \pm 8.265 ^b	150.406 \pm 4.827	8.692 \pm 0.288 ^b
	BB(3)	23.146 \pm 2.395 ^a	293.607 \pm 29.025 ^a	137.993 \pm 16.953	10.633 \pm 0.825 ^{ab}
	AB(21)	21.249 \pm 0.905 ^a	242.074 \pm 10.971 ^b	148.186 \pm 6.408	9.410 \pm 0.424 ^{ab}
	AC(5)	22.081 \pm 1.855 ^a	256.958 \pm 22.483 ^b	137.753 \pm 13.132	10.860 \pm 0.623 ^a

The data are expressed as "mean \pm SD". Consecutive letters indicate significant differences ($P < 0.05$), whereas non-consecutive letters indicate extremely significant differences ($P < 0.01$).

Table 5. Immune parameters of the different exon 4 genotypes in the 4 populations

Populations	Genotype (number)	IgG levels (g/l)	IL-10 levels (pg/ml)	IFN- γ levels (pg/ml)	WBC counts ($10^9/l$)
N-W	OO(33)	17.530 \pm 0.424	221.169 \pm 11.423	149.058 \pm 3.321	7.882 \pm 0.350
	TT(1)	16.531	202.885	130.587	6.124
	TO(18)	18.659 \pm 0.574	208.856 \pm 15.467	151.667 \pm 4.497	8.661 \pm 0.474
Y-N	OO(58)	16.449 \pm 0.352	278.316 \pm 7.443	168.544 \pm 4.943	9.407 \pm 0.269
	TO(4)	16.772 \pm 1.339	228.586 \pm 28.344	142.410 \pm 18.823	8.950 \pm 1.024
N-Y	OO(22)	16.321 \pm 0.441	248.990 \pm 9.851	181.651 \pm 8.244	10.245 \pm 0.119
	TO(2)	16.822 \pm 1.462	188.330 \pm 32.673	129.371 \pm 27.341	9.900 \pm 1.134
F-Y	OO(60)	19.800 \pm 0.552	255.551 \pm 6.513	149.031 \pm 3.716	9.034 \pm 0.233 ^b
	TT(1)	20.424	201.336	147.253	8.223
	TO(5)	22.081 \pm 1.928	256.958 \pm 22.751	137.753 \pm 12.979	10.875 \pm 0.748 ^a

The data are expressed as "mean \pm SD". Consecutive letters indicate significant differences ($P < 0.05$), whereas non-consecutive letters indicate extremely significant differences ($P < 0.01$).

He of exon 3 in the N-W and the F-Y populations was both significantly higher than that in the 2 hybrid populations. This finding suggests that the N-W and F-Y populations have relatively rich genetic diversity and certain potential selection. Exon 4 of the N-W population had the highest *He*, which indicates that N-W has more choices of selection compared with the other 3 populations. Generally, the heterozygosity of hybrid offspring is higher than those of both parents. In this study, the 2 parental populations had higher heterozygosity than the 2 hybrid populations, which may be attributed to the following reasons. First, the hybrid populations in this study had high proportions of full siblings and relatively few parents. Second, the genetic basis of offspring generated via hybridization should be enriched in theory, but the actual heterozygosity in terms of specific genes is not necessarily high.

PIC is an important indicator for gene fragment polymor-

phism. The populations had highly polymorphic or moderately polymorphic exon 3, which indicated that these 4 rabbit populations might have certain potential in the choice of exon 3 genotypes. Only the N-W population had moderately polymorphic exon 4, whereas the exon 4 of the other populations had low polymorphism. This difference indicates that N-W may be better than the other 3 populations if we want to select the genotypes on exon 4. The results above are consistent with the genetic heterozygosity.

IL-10, a multi-titer cytokine, plays an important immunoregulatory role in inflammation, cancer and autoimmune diseases. Researchers found that IL-10 gene SNPs directly affect *in vivo* IL-10 expression; thus, they are closely related to many diseases [6, 8, 13, 14, 19]. A study proved that IL-10 knockout mice are susceptible to infection with spontaneous inflammatory bowel disease [25], which shows that IL-10 maintains immune homeostasis. Thus, we speculate that the

IL-10 levels in healthy individuals may affect immunity. With the improved immunity and immune function after weaning, IgG remains relatively stable [4, 5]. Therefore, IgG is a relatively reliable index for disease resistance. IFN- γ is a relatively important immune parameter, and its levels *in vivo* reflect the resistance of individuals to some extent [12]. Therefore, studies of this parameter are important. WBCs are responsible for many important tasks in animal bodies. WBCs phagocytose foreign matter and produce antibodies and are thus related to the healing of injuries and damage, the capacity to resist pathogen invasion and resistance to diseases. Therefore, within the normal range, higher levels of immune parameters correspond to stronger disease resistance.

SNPs A1435G and G1519A on exon 3 are both synonymous mutations. We used to believe that SNPs are silent as they result in synonymous codon substitutions and that because these silent SNPs do not change the amino acid composition of the protein product, they have largely been assumed to exert no discernible effect on gene function or phenotype. But, recent studies by Kimchi-Sarfaty *et al.* have shown that silent SNPs can also affect *in vivo* protein folding and consequently function [9]. They believe that although synonymous mutation does not change the amino acid composition, it still can change the base sequence of the mRNA, which may affect the translation speed and life of mRNA itself and then change the protein expression. Furthermore, the spatial structure of this protein may also be altered. So, we discussed the association of exon 3 polymorphism with immune parameters to see whether the 2 synonymous mutations could change immune performance, even though they do not cause any amino acid changes. The results showed that BB may be a favorable genotype that is closely associated with IL-10 levels; AC may be a favorable genotype for WBC counts, and AA may be an unfavorable genotype for IgG.

About exon 4, the T base insertion early terminates the amino acid coding, but the functional domains of IL-10 gene aren't within the absent amino acids. So, even though this insertion causes frameshift mutation, the traits may not be affected. The results showed that only F-Y rabbits with genotype TO had significantly higher WBC counts than those with genotype OO, and no other significant differences were found. This phenomenon may be due to the environment, but the specific reasons need to be further studied.

In summary, we analyzed and compared the immune parameters among the genotypes on 2 exons of the IL-10 gene to determine whether the SNPs are associated with immune traits. The results show that SNPs on exon 3 are significantly associated with immune traits, while SNP on exon 4 may not significantly affect immune traits, but the mechanism is yet to be further studied.

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